Application Forum

Dynamic imaging of calcium and STIM1 in the same cell using wide-field and TIRF microscopy

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INTRODUCTION

Live-cell imaging offers the power of capturing the dynamics of biological action in live cells and in real time, something not previously available with biochemical approaches. Microscopy has progressed and static morphological observation can now be complemented by the ability to monitor dynamic cellular activities in living tissues with sub-micrometer resolution in real time. This offers valuable insight into the nature of cellular and tissue function, not previously possible using fixed cell techniques. Researchers investigating calcium signaling have particularly benefitted from live-cell imaging, and new discoveries in the field continue with new developments in imaging technology.

STIMULATING MOLECULES

Calcium signaling is involved in a plethora of cellular functions and regulating intracellular calcium concentration is a critical part of the process. Entry of calcium ions (Ca^{2+}) across the plasma membrane can replenish intracellular stores and is activated following the receptor-mediated release of calcium from the endoplasmic reticulum (ER). This store-operated calcium entry (SOCE) pathway is a well-established mechanism for replenishing internal calcium stores in many cell types [1]. Calcium conveyed via this pathway is often referred to as the calcium-release activated current (CRAC) and is mediated by plasma membrane localized CRAC or SOCE channels [2].

The CRAC channel is the best-characterized store-operated calcium (SOC) influx channel and is essential to the immune response, where sustained activity of CRAC channels is required for gene expression and proliferation. ORAI1 has recently been identified as the pore-forming subunit of CRAC [3] and the protein linking ER store filling and calcium influx via Orai1 activation has been identified as stromal interaction molecule 1 (STIM1). After release of stored calcium, STIM1 proteins detect internal store depletion and migrate to the plasma membrane from their diffuse distribution throughout the ER membrane. Here they influence the activity of several different types of calcium channels, including the SOC and CRAC channels, establishing a crucial link with the internal calcium stores [4]. A second STIM isoform, STIM2 has also been identified which, by virtue of an EF-hand that has lower calcium sensitivity, acts to regulate basal calcium entry [5].

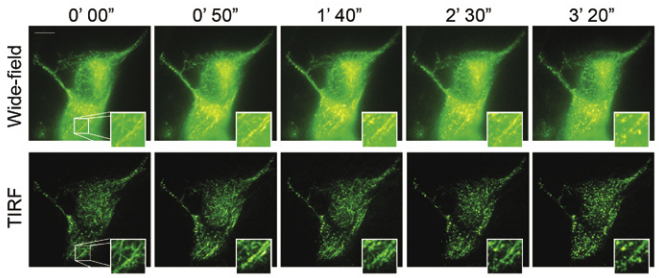

Figure 1. Comparison of wide-field epifluorescence and total internal reflection fluorescence (TIRF) images from STIM1-YFP transfected HEK cells. Thapsigargin (2 µm) was added at 0 min. Bar = 10 µm.

DYNAMIC IMAGING OF CALCIUM AND STIM1

The authors have demonstrated the ability to near-simultaneously monitor intracellular calcium along with two different fluorescent proteins in the same cell using live-cell imaging. Here they describe how a combination of advanced wide-field fluorescence imaging and total internal reflection fluorescence microscopy (TIRFM) has been used for live-cell microscopy. This fluorescence imaging approach allows the rapid multi-dimensional analysis of fluorescently labeled cells, which has permitted the dynamic imaging of calcium and STIM1 from the same cell.

MATERIALS AND METHODS

Live-cell imaging

Human embryonic kidney (HEK)-293 cells were transfected with YFP-/mCherry-tagged constructs 16 h prior to imaging. For calcium imaging, cells were loaded with Fura-2 AM (2 µM) diluted in extracellular calcium buffer for 30 min then washed with extracellular calcium buffer and left

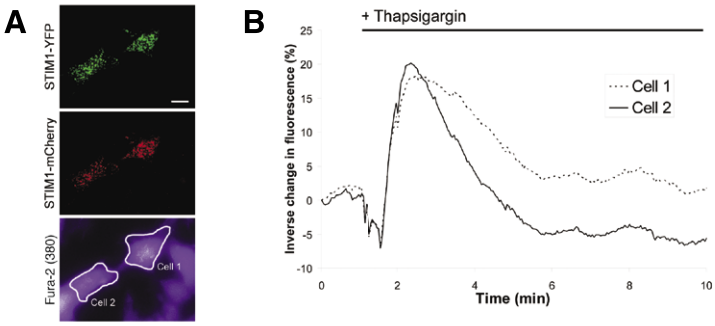

Figure 2. Acquisition of TIRF images and wide-field epifluorescence images allows STIM1 translocation to be monitored concomitant with changes in intracellular calcium concentration. (A) Sequential acquisition of STIM1-YFP (TIRF), STIM1-mCherry (TIRF), and Fura-2 (wide-field epifluorescence) images from the same cells. A set of three images (2× TIRF, 1× wide-field) was acquired every 2 s (images shown were acquired 5 min after application of thapsigargin) (B) Change in intracellular calcium concentration of the two cells outlined in A after the application of thapsigargin (2 µm). Bar = 10 µm.

for a period of 30 min to allow for hydrolysis of the lipophilic blocking groups. Coverslips were mounted in stainless steel rings, and cells were immersed in extracellular calcium buffer for imaging. To elicit STIM1 translocation, thapsigargin (2 µM) was added in calcium-free extracellular buffer (as for calcium buffer, except 1 mM EGTA replaces $CaCl_2$). By inhibiting the ER calcium pump, thapsigargin uncovers a passive leak of calcium from the ER, which results in an increase in cytosolic calcium concentration. Store-depletion secondarily activates plasma membrane calcium channels, allowing an influx of calcium into the cytosol.

All images were acquired using an Olympus Cell^R imaging system comprising IX81 microscope, 100× 1.45 NA apochromat objective, MT-20 illumination unit, 488 nm/20 mW and 561 nm/25 mW lasers, Hamamatsu ORCA ER camera. The IX81 microscope was fitted with a Solent Scientific environment chamber to maintain temperature at 32°C. Wide-field epifluorescence images of YFP and Fura-2 were acquired using a 470/40 excitation filter and 505 long pass filter and a 380/15 excitation and 505 long pass filter, respectively. TIRF images of YFP and mCherry were acquired using 488 nm and 561 nm laser excitation, respectively, and a 527/21 645/24 dual bandpass emission filter.

RESULTS

By employing a highly advanced fluorescence imaging system and exploiting specific fluorescent probes, TIRF illumination was used to observe cellular structures at the plasma membrane decorated with fluorescently tagged STIM-1 near-simultaneously with intracellular calcium. Figure 1 compares both wide-field epifluorescence and TIRF images from STIM1-YFP transfected HEK cells. TIRF microscopy is the method of choice for high-resolution imaging of STIM1 translocation/redistribution at the plasma membrane as images are not compromised by out-of-focus blur. Acquisition of TIRF images and wide-field epifluorescence images (Figure 2) allows STIM1 translocation to be monitored concomitant with changes in intracellular calcium concentration. Figure 2A shows the sequential acquisition of STIM1-YFP (TIRF), STIM1-mCherry (TIRF), and Fura-2 (wide-field epifluorescence) images from the same cells. A set of three images (2× TIRF, 1× wide-field) were acquired every 2 s (images shown were acquired 5 min after application of thapsigargin). Figure 2B illustrates the change in intracellular calcium concentration of the two cells outlined in Panel A after the application of thapsigargin.

CONCLUSIONS

Using an Olympus Cell^R imaging system, it is possible to monitoring the dynamic processes of calcium signalling and STIM1 translocation within the same cell. This may provide new insights into the potential role of how STIM proteins are necessary for the signaling process of Ca^{2+} store depletion to the induction of Ca^{2+} influx. Furthermore, this technology can be used to further investigate how STIM proteins function as Ca^{2+} sensors, and also how this process may affect local sites near the plasma membrane and ER.

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